SCIENCE CHINA
Life Sciences

SPECIAL TOPIC: Haunted with and hunting for viruses
• REVIEW •

August 2013 Vol.56 No.8: 697–700
doi: 10.1007/s11427-013-4518-9

An emerging hemorrhagic fever in China caused by a novel bunyavirus SFTSV

ZHANG XiaoShuang[1], LIU Yan[2,3], ZHAO Li[1], LI Bing[4], YU Hao[2], WEN HongLing[1] & YU Xue-Jie[1,2*]

[1]*School of Public Health, Shandong University, Jinan 250012, China;*
[2]*University of Texas Medical Branch, Galveston, Texas 77555-0609, USA;*
[3]*Anhui Province Center for Disease Control and Prevention, Hefei 230601, China;*
[4]*Jinan Junqu Center for Disease Control and Prevention, Jinan 250014, China*

Received May 28, 2013; accepted June 16, 2013

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging hemorrhagic fever in rural areas of China and is caused by a new bunyavirus, SFTSV, named after the disease. The transmission vectors and animal hosts of SFTSV are unclear. Ticks are the most likely transmission vectors and domestic animals, including goats, dogs, and cattle, are potential amplifying hosts of SFTSV. The clinical symptoms of SFTS are nonspecific, but major symptoms include fever, gastrointestinal symptoms, myalgia, dizziness, joint pain, chills, and regional lymphadenopathy. The most common abnormalities in laboratory test results are thrombocytopenia (95%), leukocytopenia (86%), and elevated levels of serum alanine aminotransferase, aspartate aminotransferase, creatine kinase, and lactate dehydrogenase. The fatality rate for SFTS is 12% on average, and the annual incidence of the disease is approximately five per 100000 of the rural population.

SFTSV, tick, thrombocytopenia, leukopenia, bunyavirus, *Phlebovirus*, hemorrhagic fever, emerging infectious diseases

Citation: Zhang X S, Liu Y, Zhao L, et al. An emerging hemorrhagic fever in China caused by a novel bunyavirus SFTSV. Sci China Life Sci, 2013, 56: 697−700, doi: 10.1007/s11427-013-4518-9

In June 2009, an outbreak of an unknown infectious disease occurred in rural areas of Hubei Province, China, affecting 17 patients, five of whom died. A rickettsiologist, Dr. Yu Xue-Jie, was asked by the Chinese Center for Disease Control and Prevention (China CDC) to investigate the potential etiological pathogen of the disease, which was suspected to be human anaplasmosis (HA) [1]. After careful analysis of the patients' clinical information, Dr. Yu concluded that the patients' symptoms were inconsistent with HA because most had gastrointestinal symptoms, such as nausea, vomiting, and diarrhea, which are rarely observed in HA patients [2]. Moreover, Dr. Yu's laboratory detected no antibodies directed against the HA pathogen, *Anaplasma phagocytophilum*, or its DNA in any of the 17 patients. Therefore, Dr. Yu considered that the disease was not HA, but was more likely to be of viral origin, although previous studies had failed to identify a viral pathogen. A strategy was designed to isolate the etiological agent using multiple cell lines, including DH82, Vero, Vero E6, THP-1, and IS6 cells, in an attempt to isolate *A. phagocytophilum*, *Ehrlichia*, *Rickettsia*, or a virus. After inoculation with a blood sample from a patient in June 2009, a cell cytopathic effect (CPE) was observed in DH82 cells and the virus was observed with electron microscopy. The genomic sequence indicated that the agent was a novel virus, most closely related to *Phlebovirus* in the family Bunyaviridae [3]. After the identification of the virus, the China CDC undertook a surveillance program in 2010 and identified viral infections in six provinces of east China [2]. The virus was first designated "DBM virus", because it was isolated from a patient living in the

*Corresponding author (email: yuxuejie@sdu.edu.cn)

DaBie Mountain area [4], but was renamed severe fever with thrombocytopenia syndrome virus (SFTSV) by the China CDC [3,5]. Similar viruses have recently been identified in both the United States and Japan [6].

1 SFTSV and its molecular biology

Electron microscopy showed that SFTSV is spherical, with a diameter of 100 nm (Figure 1). The virions were observed inside vacuoles, presumably in the Golgi apparatus [3]. SFTSV is sensitive to acid, heat, and chemicals such as ether and sodium deoxycholate [7].

The genome of SFTSV consists of three RNA segments: the large (L), medium (M), and small (S) segments [3]. The L segment contains 6368 nucleotides, with one open reading frame encoding 2084 amino acids. The M segment contains 3378 nucleotides, with one open reading frame encoding a 1073-amino-acid glycoprotein (Gn and Gc) precursor. The S segment contains 1744 nucleotides of ambisense RNA, encoding two proteins, the N and NSs proteins, in opposite orientations, separated by a 62-bp intergenic region. The 3′ and 5′ ends are complementary and can form a panhandle structure. The S segment is relatively conserved between SFTSV and other phleboviruses, with 41% amino acid sequence homology, whereas the L and M segments are less conserved, with only 21%–36% amino acid sequence homology between SFTSV and the other phleboviruses [3]. SFTSV is most closely related to the Heartland virus, recently isolated from patients in the USA, with nearly 70% RNA sequence homology. SFTSV is next closely related to the Uukuniemi virus and Bhanja virus [3,8,9].

Phylogenetic, evolutionary, and structural analyses of all available SFTSV genomic sequences revealed recombination of SFTSV in the M and L segments [10]. A mosaic L segment sequence, which has descended from two major circulating lineages of SFTSV in China, represents the first evidence that homologous recombination plays a role in SFTSV evolution. Selection analyses indicated that negative selection is predominant in the SFTSV genes, yet the differences in the selective forces among genes are consistent with those of the other *Phlebovirus* species.

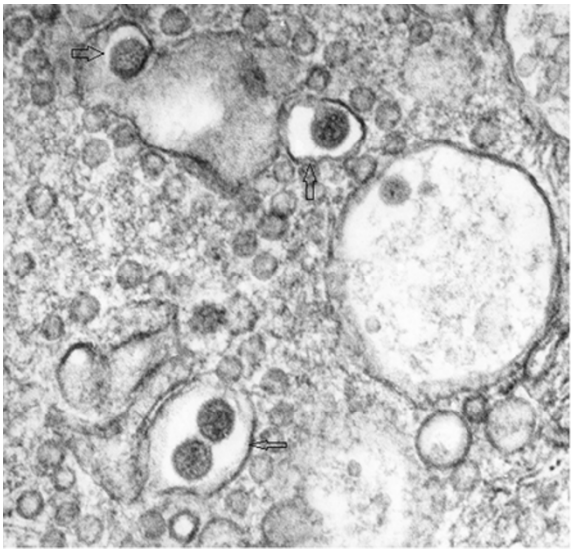

Figure 1 Hemorrhage in the mouth of a patient with SFTS.

2 Transmission route, vectors, and animal hosts of SFTSV

Except for Hantavirus, the bunyavirusesare usually maintained in arthropod vectors by transovarial transmission, whereas their vertebrate hosts may act as amplifying hosts [11]. SFTSV RNA has been detected in the ticks *Haemaphysalis longicornis* and *Boophilus microplus* collected from domestic animals, including cattle, goats, and dogs [3,12]. It has also been detected by PCR in *Leptotrombidium scutell* mites collected from *Apodemusagrarius* mice, in *Laelaps echidnina* mites collected from *A. agrarius* and goats in Jiangsu Province [13], and in the gadfly in Henan Province [14]. SFTSV was not detected in mosquitoes [3] and has not yet been investigated in the sandfly. SFTSV has not been detected in tick eggs and has been detected in only tick collected from vegetation [12]. The arthropods positive for SFTSV suggest that they could be vectors of SFTSV or that they could acquire SFTSV from infected animals. Therefore, these arthropods should be further investigated as vectors of SFTSV.

The seroprevalence of SFTSV has been investigated in domestic animals, including goats, dogs, cattle, pigs, and chickens. In Shandong Province, 83%–95% of goats were positive for SFTSV antibodies [15–17]; in Jiangsu Province, 57% of goats, 32% of cattle, 6% of dogs, 5% of pigs, and 1% of chickens were seropositive for SFTSV [17]; and in Hubei Province, 55.0% (6/11) of dogs, 36.7% (2/3) of goats, and 80.0% (4/5) of cattle were seropositive for SFTSV [18]. However, only a small proportion of the animals studied (1.7%–5.3%) were found to carry low levels of viral RNA in their sera [19]. This suggests that the domesticated animals act as amplifying hosts of SFTSV and play a major role in feeding the ticks that spread SFTSV. Rats were seropositive for SFTSV in Jiangsu Province, at a rate of 6.9% in wild rodents and 7.87% in house rodents [20]. The seroprevalence of SFTSV in other wild animal species has not been investigated.

3 Nosocomial transmission of SFTSV

The nosocomial transmission of SFTSV has been demonstrated in several studies [21–24]. The earliest confirmed nosocomial transmission occurred in 2006 in Anhui Province [21], when SFTSV was most likely transmitted from person to person through contact with the patient's blood

[21]. The incubation period of nosocomial transmission is between six and 13 days after contact with or exposure to the blood of an index patient [21].

4 Geographical distribution and seasonal outbreaks

SFTS patients have been identified in 16 provinces of east China [25] (Figure 2). SFTS has occurred most frequently in central China, especially in Henan, Hubei, and Shandong Province, which have reported 48.2%, 21.9%, and 15.7% of the total SFTS cases, respectively [26]. These cases of SFTS were sporadic and occurred predominantly in hilly areas. SFTS occurred from April to November in different areas of China and 96.0% (148/154) of laboratory-confirmed cases occurred nationally between May and July [3].

5 Susceptible population

The ages of SFTS patients ranged from 1 to 90 years, with a median age of 58 years [26]. People aged over 50 years constituted 75% of cases but only 26% of the population under surveillance (P<0.001). Fifty-six percent of cases were female, although they constitute only 49% of the total population (P=0.029), and 97% of cases were farmers living in wooded and hilly areas and working in the fields. Precise information about the occupations of the total population under surveillances was not available [27]. SFTSV mainly infected older persons, which suggests that SFTSV infects people with low immunity.

Serosurveillance indicated that 1.0%–3.8% of the population in the hilly areas of China were positive for SFTSV antibodies [15–17], suggesting that although a large population was infected with SFTSV, a very small proportion of infected individuals developed the disease. The incidence of SFTS ranged from $0.33/10^4$ in Hubei Province to $5/10^5$ in Shandong Province [18] (unpublished).

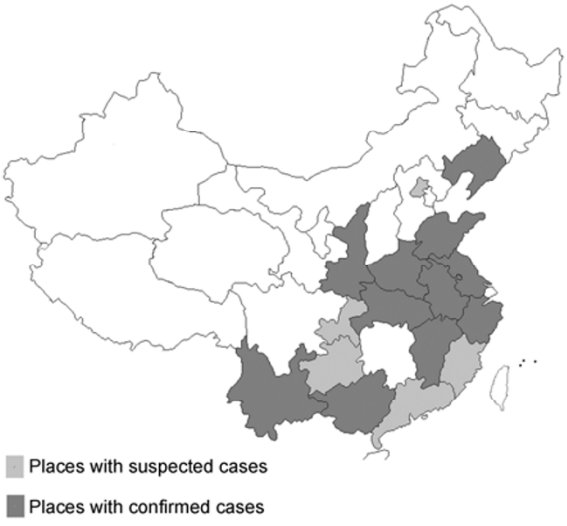

Figure 2 The provinces in China where SFTSV and SFTS patients were identified until 2012.

6 Clinical symptoms and diagnosis of SFTS

The clinical symptoms of SFTS are nonspecific, but the major symptoms include fever, gastrointestinal symptoms, myalgia, and regional lymphadenopathy. Dizziness (31.3%), joint pain (25%), and chills (18.8%) are also common in SFTSV-infected patients, and hemorrhage in mouth is also frequently observed. The most common abnormalities on laboratory tests are thrombocytopenia (95%) and leukocytopenia (86%). Proteinuria (in 84% of patients) and hematuria (in 59%) are also observed [3]. Multiple organ failure develops rapidly in most patients, as evident in their elevated levels of serum alanine aminotransferase, aspartate aminotransferase, creatine kinase, and lactate dehydrogenase. The case fatality rate of SFTS was about 11%–12% in 2009 and 2010, with one study reporting 21 deaths in 171 confirmed cases from the whole country and another study reporting 21 deaths in 188 cases in Hubei Province in 2010 [3,18]. The national mortality rate was 6.3% (129/2047) in 2011 and 2012 [26]. The reason for the improvement in the mortality rate from 2010 to 2012 is unclear, but might have resulted from the different sample sizes and/or the early diagnosis and proper treatment of patients after the identification of SFTSV. Patients with SFTS have severe clinical symptoms, and progress rapidly to multiple organ dysfunction syndrome (MODS), with a high fatality rate of 12%–30% [3]. Multiorgan failure is associated with death and the SFTS patients with MODS who died were older and had lower platelet counts than those who survived [28]. Mortality was significantly higher in misdiagnosed SFTS patients than in patients with confirmed SFTS (16% vs. 6.1%, respectively) [28]. The treatment for SFTS is nonspecific and the results of treatment are difficult to evaluate because there have been no cohort studies.

The diagnosis of SFTSV infection is based on the patient's clinical manifestations, such as fever, thrombocytopenia (and/or leukopenia), and elevated levels of liver enzymes, and on their living and working environments, specifically in rural areas with forests and shrubs. Confirmation of SFTSV infection requires one of the following conditions: isolation of SFTSV from the patient's serum, detection of SFTSV RNA in the patient's serum by PCR, or detection of SFTS antibodies in the patient's serum. Several methods have been developed to amplify the viral RNA, including reverse transcription-PCR (RT-PCR) and RT-loop-mediated isothermal amplification (RT-LAMP) [29–32]. Amplification of the S segment is the most sensitive RT-PCR assay [29]. A simple near-instrument-free molecular method that incorporates RT-cross-priming amplification (RT-CPA) coupled to a vertical-flow visualization strip has also been

developed for the rapid detection of SFTSV [32]. To detect viral antibodies, an enzyme-linked immunosorbent assay (ELISA) has been developed using a recombinant N protein of SFTSV [17]. Virus isolated from blood is mainly used in research laboratories and CDCs. The SFTSV virus can be isolated using DH82 cells, which are round monocytes before infection but attach to the culture flask after infection with the virus [3]. SFTSV exerts a CPE on DH82 cells but not on Vero cells or Vero E6 cells in primary isolation, which may be why isolation of SFTSV was failed for such a long time in China, because Vero E6 cells were used in the past. A serological test for the diagnosis of SFTSV infection could be developed using immunofluorescence or ELISA [3].

7 Control and prevention of SFTSV

SFTS is a severe disease, with a high case fatality rate, that occurs in areas of China with high population densities. The primary hosts for *H. longicornis* in China are domestic animals, including goats, sheep, cattle, and dogs. Controlling ticks on these animals with chemicals is essential to reducing the tick population and to reducing SFTSV infections in humans. No vaccine for SFTSV has yet been developed.

1 Zhang L, Liu Y, Ni D, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. JAMA, 2008, 300: 2263–2270
2 Dumler J S, Choi K S, Garcia-Garcia J C, et al. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. Emerg Infect Dis, 2005, 11: 1828–1834
3 Yu X J, Liang M F, Zhang S Y, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. N Engl J Med, 2011, 364: 1523–1532
4 Stone R. Rival teams identify a virus behind deaths in central China. Science, 2010, 330: 20–21
5 National Guideline for Prevention and Control of Severe Fever with Thrombocytopenia Syndrome. Beijing: Ministry of Health of People's Republic of China, 2010, 2013
6 Wu Y, Gao G F. Severe fever with thrombocytopenia syndrome virus expands its borders. Emerg Microbes Infect, 2013, in press
7 Li D. Fever with thormbocytopenia associated with a novel *Bunyavirus* in China. Chin J Exp Clin Virol, 2011, 25: 81–84
8 McMullan L K, Folk S M, Kelly A J, et al. A new phlebovirus associated with severe febrile illness in Missouri. N Engl J Med, 2012, 367: 834–841
9 Matsuno K, Weisend C, Travassos da Rosa A P, et al. Characterization of the Bhanja serogroup viruses (Bunyaviridae): a novel species of the genus *Phlebovirus* and its relationship with other emerging tick-borne phleboviruses. J Virol, 2013, 87: 3719–3728
10 He C Q, Ding N Z. Discovery of severe fever with thrombocytopenia syndrome bunyavirus strains originating from intragenic recombination. J Virol, 2012, 86: 12426–12430
11 Walter C T, Barr J N. Recent advances in the molecular and cellular biology of bunyaviruses. J Gen Virol, 2011, 92(Pt 11): 2467–2484
12 Zhang Y Z, Zhou D J, Qin X C, et al. The ecology, genetic diversity, and phylogeny of Huaiyangshan virus in China. J Virol, 2012, 86: 2864–2868
13 Wang Q K, Ge H M, Li Z F, et al. Vector research of severe fever with thrombocytopenia syndrome virus in gamasid mites and chigger mites. Chin J Vector Biol Contr, 2012, 23: 452–454
14 Liu Y, Huang X Y, Du Y H, et al. Survey on ticks and detection of new bunyavirus in some vect in the endemic areas of fever, thormbocytopenia and leukopenia syndrome in Henan Province. Chin J Prevent Med, 2012, 46: 500–504
15 Zhao L, Zhai S, Wen H, et al. Severe fever with thrombocytopenia syndrome virus, Shandong Province, China. Emerg Infect Dis, 2012, 18: 963–965
16 Cui F, Cao H X, Wang L, et al. Clinical and epidemiological study on severe fever with thrombocytopenia syndrome in Yiyuan County, Shandong Province, China. Am J Trop Med Hyg, 2013, 88: 510–512
17 Jiao Y, Zeng X, Guo X, et al. Preparation and evaluation of recombinant severe fever with thrombocytopenia syndrome virus (SFTSV) nucleocapsid protein for detection of total antibodies in human and animal sera by double antigen sandwich ELISA. J Clin Microbiol, 2011, 50: 372–377
18 Liu L, Guan X H, Xing X S, et al. Epidemiologic analysis on severe fever with thrombocytopenia syndrom in Hubei Province, 2010. Chin J Epidemiol, 2012, 33: 168–172
19 Li D. A highly pathogenic new bunyavirus emerged in China. Emerg Microbes Infect, 2012, 2: e1
20 Ge H M, Wang Q K, Li Z F, et al. Survey of SFTSV carrying situation in rodents in SFTS epidemic area of Donghai county. Jiangsu Prevent Med, 2012, 23: 16–18
21 Liu Y, Li Q, Hu W, et al. Person-to-person transmission of severe fever with thrombocytopenia syndrome virus. Vector Borne Zoonotic Dis, 2011, 12: 156–160
22 Bao C J, Guo X L, Qi X, et al. A family cluster of infections by a newly recognized bunyavirus in eastern China, 2007: further evidence of person-to-person transmission. Clin Infect Dis, 2011, 53: 1208–1214
23 Chen H, Hu K, Zou J, et al. A cluster of cases of human-to-human transmission caused by severe fever with thrombocytopenia syndrome bunyavirus. Int J Infect Dis, 2013, 17: e206–e208
24 Lam T T, Liu W, Bowden T A, et al. Evolutionary and molecular analysis of the emergent severe fever with thrombocytopenia syndrome virus. Epidemics, 2013, 5: 1–10
25 Ding F, Zhang W, Wang L, et al. Epidemiologic features of severe fever with thrombocytopenia syndrome in China, 2011-2012. Clin Infect Dis, 2013, 56: 1682–1683
26 Gai Z, Liang M, Zhang Y, et al. Person-to-person transmission of severe fever with thrombocytopenia syndrome bunyavirus through blood contact. Clin Infect Dis, 2012, 54: 249–252
27 Xiong W Y, Feng Z J, Matsui T, et al. Risk assessment of human infection with a novel bunyavirus in China. WPSAR, 2012, 3, doi: 10.5365/wpsar.2012.3.4.002
28 Jie S H, Zhou Y, Sun L P, et al. Close correlation between development of MODS during the initial 72 h of hospitalization and hospital mortality in severe fever with thrombocytopenia syndrome. J Huazhong Univ Sci Technol (Med Sci), 2013, 33: 81-85
29 Chi Y Y, Zhai S Y, Wen H L, et al. SFTSV RNA detection in seropositive patients. J Shandong Univ (Health Sci), 2012, 50: 118–121
30 Sun Y, Liang M, Qu J, et al. Early diagnosis of novel SFTS bunyavirus infection by quantitative real-time RT-PCR assay. J Clin Virol, 2012, 53: 48–53
31 Yang G, Li B, Liu L, et al. Development and evaluation of a reverse transcription loop-mediated isothermal amplification assay for rapid detection of a new SFTS bunyavirus. Arch Virol, 2012, 157: 1779–1783
32 Cui L, Ge Y, Qi X, et al. Detection of severe fever with thrombocytopenia syndrome virus by reverse transcription-cross-priming amplification coupled with vertical flow visualization. J Clin Microbiol, 2012, 50: 3881–3885